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Alain RIVAL, Estelle JALIGOT, Thierry BEULE, Frédérique ABERLENC-BERTOSSI, Pascal ILBERT, Frédérique RICHAUD and James TREGEAR

Innovation and scaling-up in micropropagation: lessons from the oil palm experience

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Abstract
In the mid-seventies, the first results obtained by the strategy of reciprocal recurrent selection (RRS) of oil palm (Elaeis guineensis Jacq) and the emergence of the methods of cloning by in vitro culture led to the development of a technique of micropropagation through somatic embryogenesis which was tested initially in Côte d'Ivoire, then in Malaysia and Indonesia. This work established the utility of clonal micropropagation which was found to enable the production of high yielding clones. In addition, this development phase highlighted the difficulties related to scaling-up in relation to, on the one hand, mass production required to meet the needs of planters and, on the other hand, the genetic fidelity of the regenerated plant material. These two concerns led us to look further into the underlying mechanisms involved in somatic embryogenesis and the somaclonal variation events induced by the regeneration techniques. The development of a regeneration protocol based on the use of embryogenic suspensions has generated a method which allows production on a large scale of single somatic embryos. This method is now widely used for commercial micropropagation and it is currently field tested by several companies. In order to tackle the problem of the mantled flowering abnormality which is induced during the oil palm micropropagation process we have carried out studies of gene expression in tissue cultures as a means of establishing an early clonal conformity testing procedure. More, our studies on genomic DNA methylation changes induced by tissue culture suggest that the latter may play a key role in the determination of the mantled abnormality. We demonstrated, by the use of two complementary methods for evaluating methylation rates at the genome-wide level, that there is a highly significant DNA hypomethylation in leaves of abnormal regenerants and calli, compared to their normal counterparts. New investigations are now aimed at elucidating the mechanisms and/or sequences through which epigenetic misregulation could provoke the onset of the mantled phenotype in oil palm. Our experience in oil palm micropropagation revealed the pivotal importance of the pilot stage in the identification of both biological and technological bottlenecks for the large scale production. Once transformed in adequate questions to research, the identified problems are able to stimulate innovation and improve our knowledge on the biology and physiology of palms.

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Le Palmier à huile

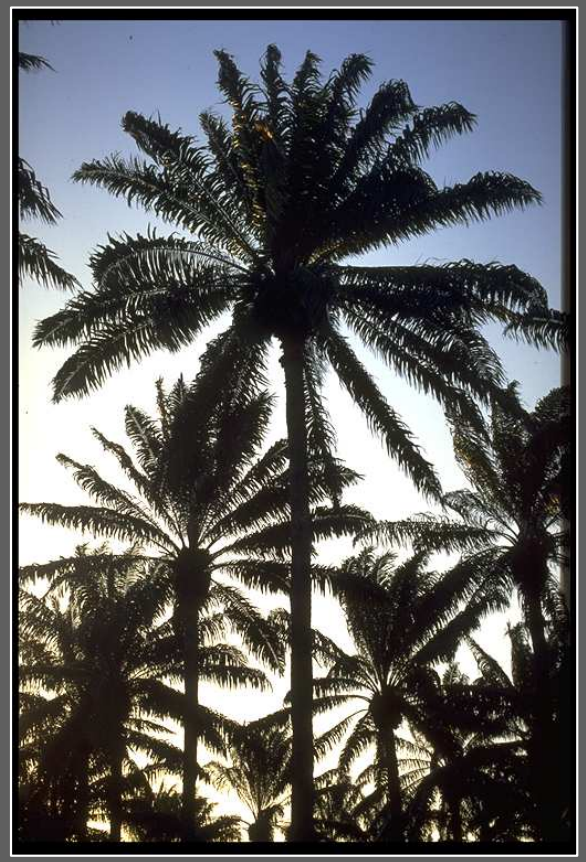
- Une herbe géante pérenne:
  Monocotyledone, *Arecaceae*

  Cocotier, dattier, rotin, chou palmiste…

- Deux espèces cultivées:
  - *Elaeis guineensis*
  - *Elaeis oleifera*

  (enrichi en acides gras insaturés)
  - Hybride interspécifique

Le palmier à huile



✓ 11 millions hectares plantés en zone intertropicale humide

✓ La première source mondiale d'huile végétale (devant le soja)

✓ Principaux pays producteurs: Indonésie et Malaisie (85%)

✓ Principaux importateurs: Chine, Inde, UE, Pakistan

Une filière stratégique pour les pays tropicaux

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Micropropagation clonale du palmier à huile

Un programme de recherche collaboratif
IRD-CIRAD

Micropopropagation clonale: scaling-up

- Faisabilité du procédé d'embryogenèse somatique
  - 2 millions de vitroplants produits
  - Un progrès génétique réel
- Transfert de technologie vers les pays producteurs: Indonésie, Malaisie, Côte d'Ivoire, Costa Rica, Colombie
- Limitations au changement d'échelle:
  - ➢ Couts de production: 2 à 4 US$ par vp (5 à 7 x semence améliorée)
  - ➢ Fidélité génétique

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Le développement pilote génère des questions de recherche

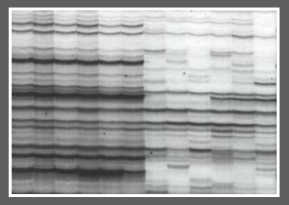
1. Les coûts de production:

Mettre en place un procédé d'embryogénèse somatique plus performant

- Production à grande échelle (10^5 vitroplants / an / clone)
- Réduction significative des couts de main-d'oeuvre

2. La fidélité génétique

Développer des marqueurs ADN/ARN/sérologiques



- pilotage du procédé de production par ES
- Controle qualité: détection précoce des variants

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PROLIFERATION
Liquid medium

Proliferation of meristematic and embryogenic clumps

PRETREATMENT
Liquid medium

Expression of somatic embryogenesis

MATURATION
Solid medium

Differentiation of somatic embryos

DEVELOPMENT
Solid medium

Shoot and root development

Subculture 1 month

1 month

1 month

1.5 months

washing

sieving & plating

subculture

Dehydratation
Encapsulation
Conservation

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La variation somaclonale “mantled”

Impact de la variation somaclonale “mantled”

	palmiers observés	palmiers normaux	“mantled” léger	“mantled” grave
IDEFOR Côte d’Ivoire	29,415	90.3%	3.7 %	6.0 %
FELDA Malaisie	18,935	92.0%	5.6 %	2.4 %
IOPRI Indonésie	6,771	87.3%	5.3 %	7.4 %

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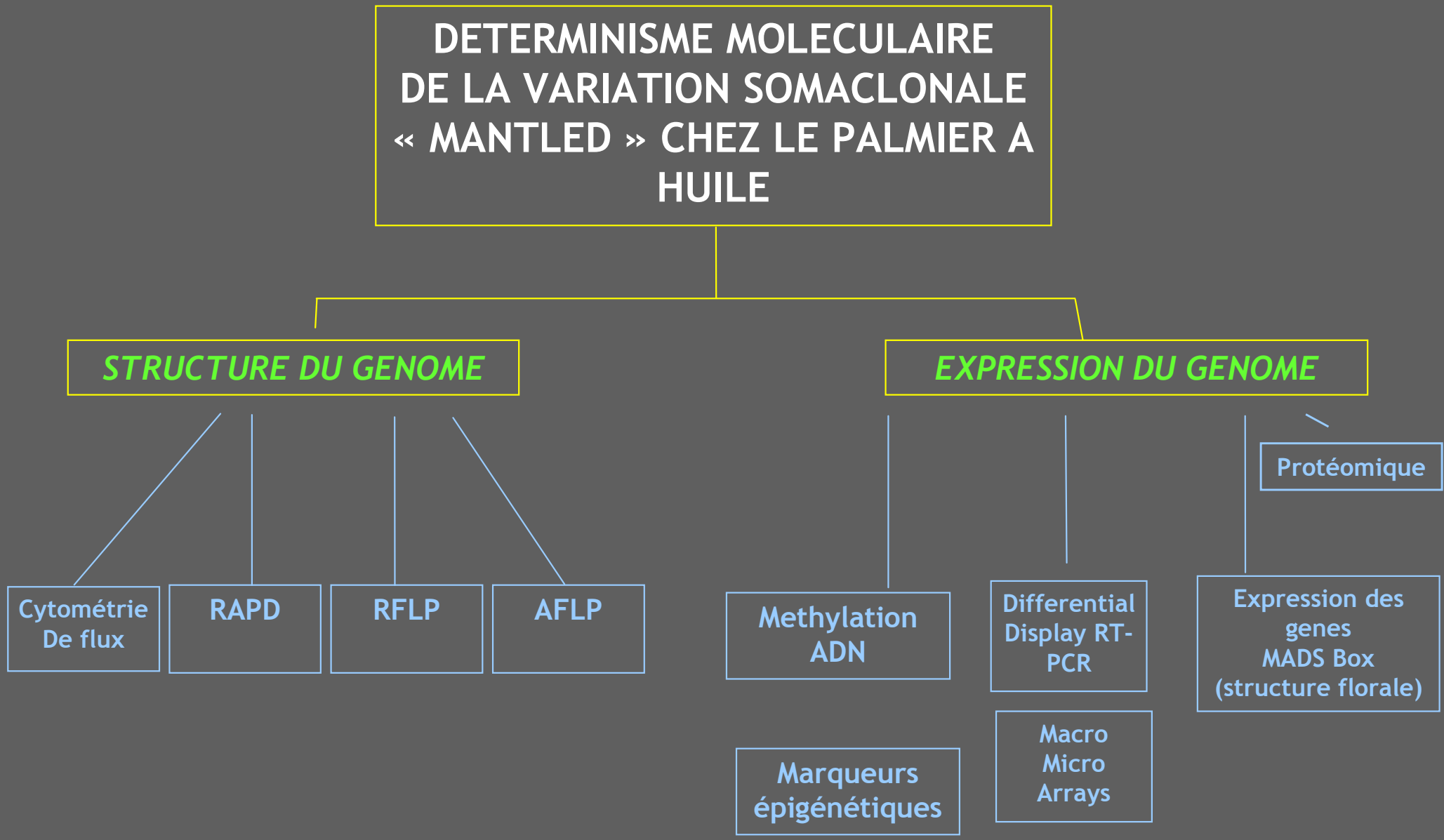
Stratégie de recherche



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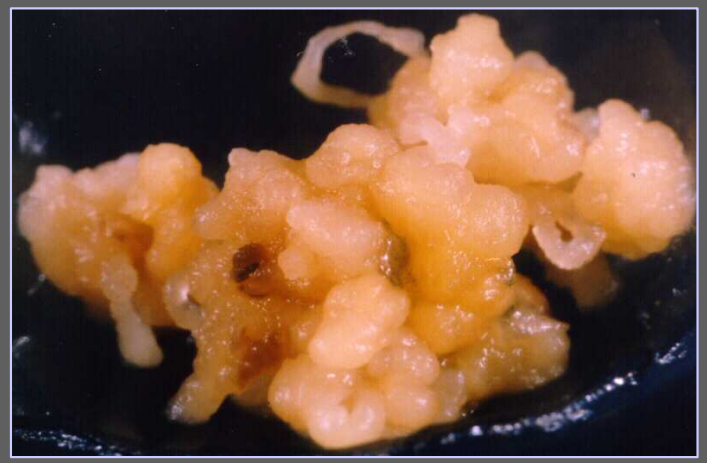
Un modèle d'étude

Cals embryogènes de palmier à huile

Cals Compacts Nodulaires



Cals à Croissance Rapide

Régénération *in vitro* par embryogenèse somatique

VARIANTS SOMACLONAUX
< 5%

VARIANTS SOMACLONAUX
100%

Quels sont les mécanismes moléculaires responsables ?

- ✓ Niveau de ploidie
- ✓ Marqueurs ADN
  - RAPDs
  - AFLPs
- ✓ Expression différentielle du génome
  - ddRT-PCR / Puces ADN
  - Homeotic MADS Box RFs
- ✓ Marqueurs épigénétiques
  - ✓ Taux de methylation globale
  - ✓ RFLP/AFLP Methylation-sensibles
  - ✓ DNA methyltransferases
  - ✓ Restructuration de la Chromatine
  - ✓ Petits ARNs non codants

Publications majeures

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RIVAL A. , E. JALIGOT, T. BEULÉ & J. FINNEGAN (2008) Isolation and differential expression of MET, CMT and DRM methyltransferase genes from oil palm (*Elaeis guineensis* Jaq.) in relation with the “mantled” somaclonal variation. *Journal of Experimental Botany* 59(12): 3271–3281.

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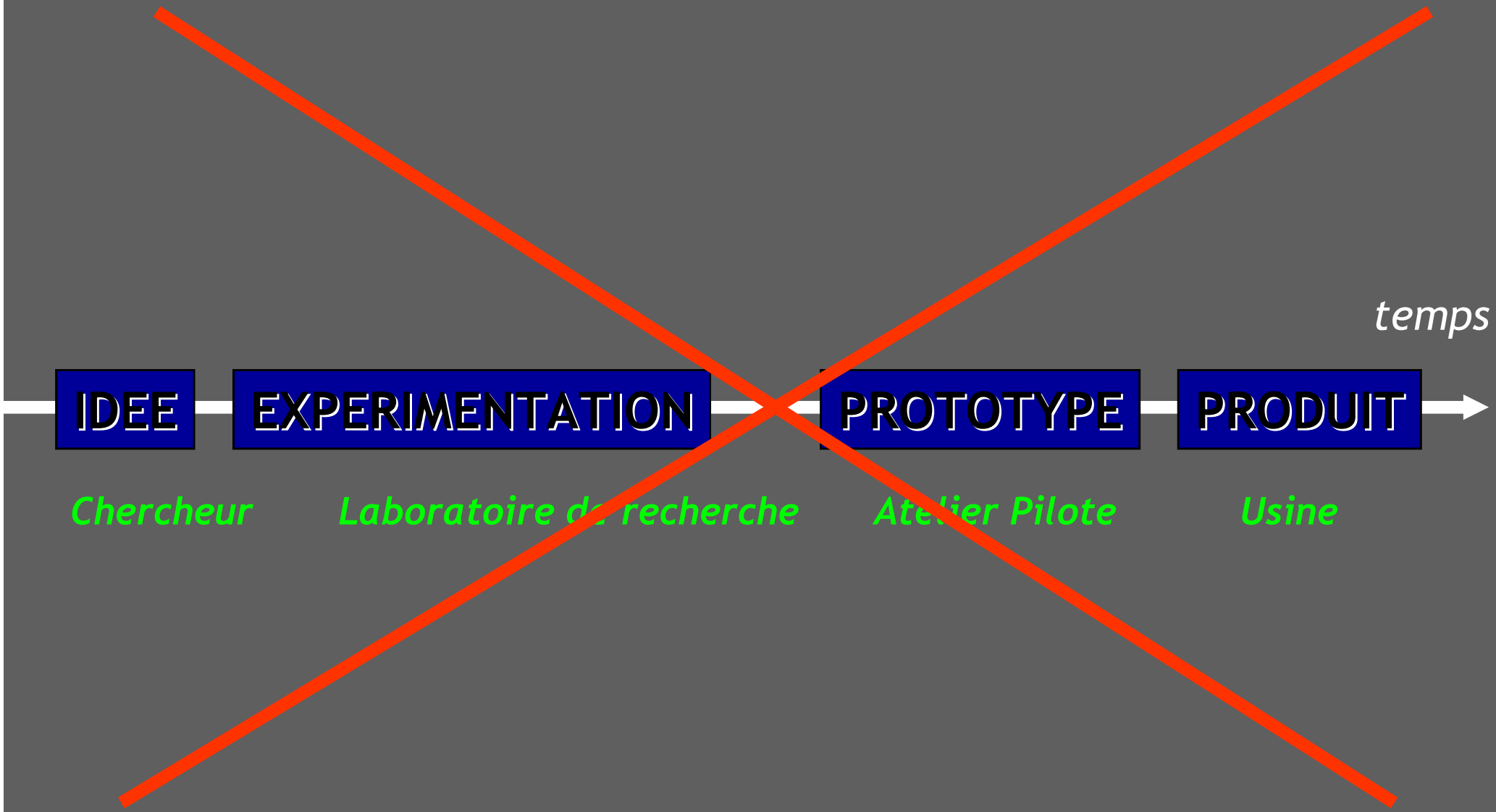
L'innovation n'est pas un processus linéaire …



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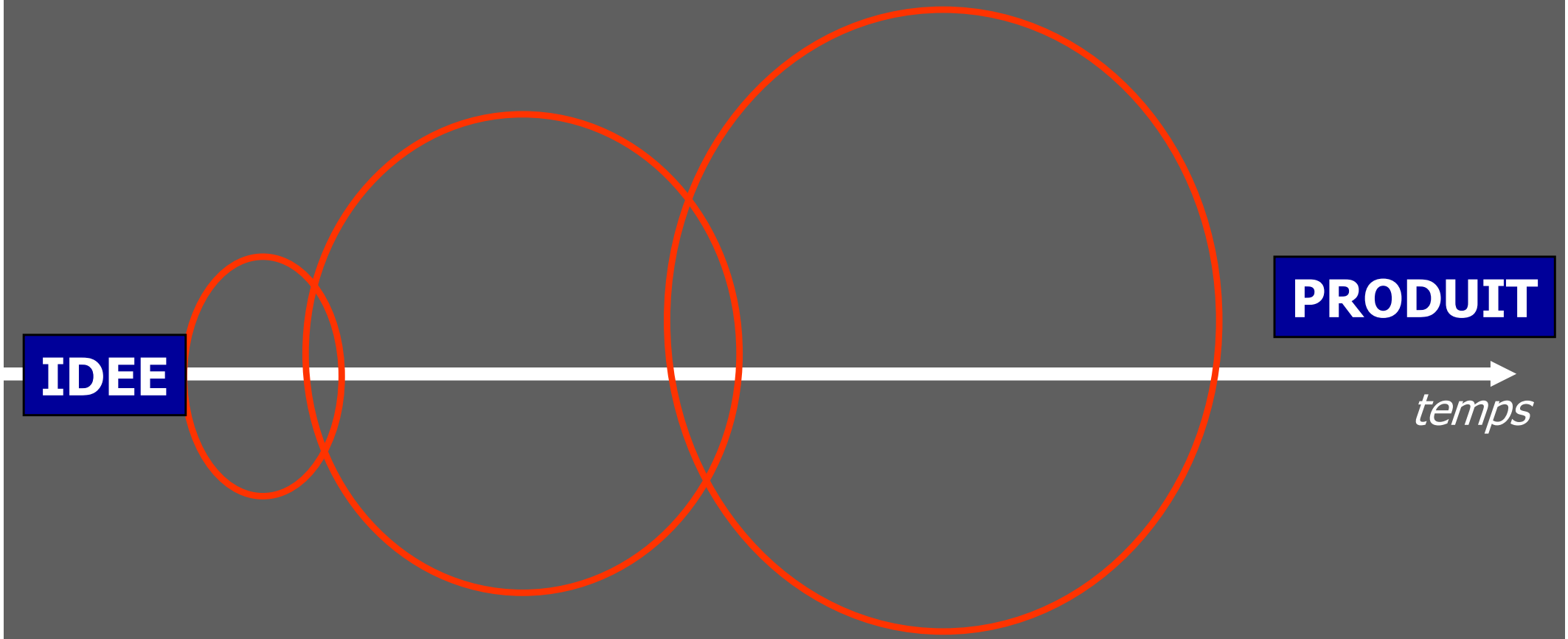
L'innovation suit une série de boucles imbriquées …



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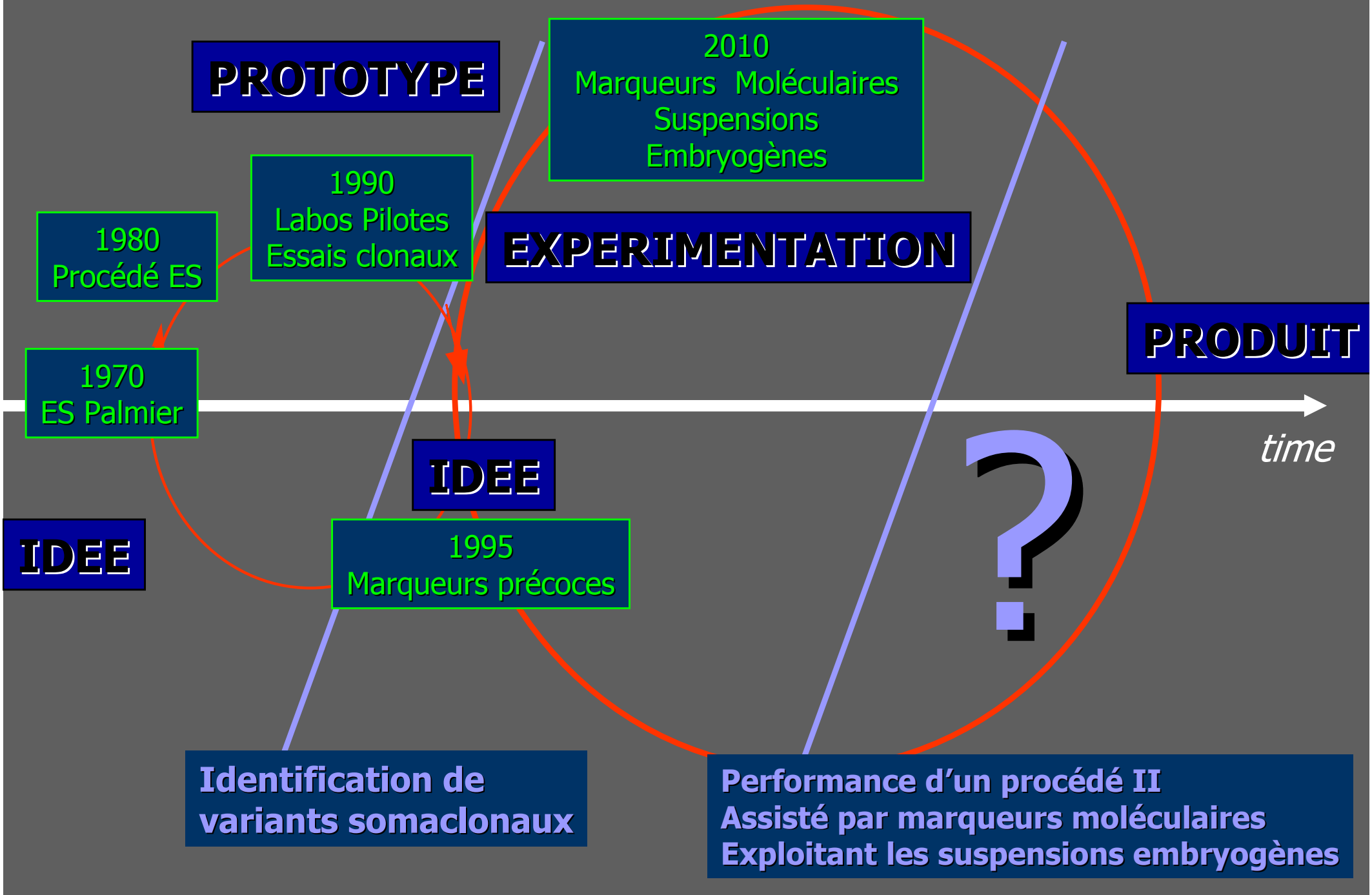
Micropropagation du palmier à huile: un cas d'étude



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Merci de votre attention …